associated with severe cardiac hypertrophy. We observed a heart weight to body weight ratio of 4.4 ± 0.1, 4.9 ± 0.1, 8.9 ± 0.3 and 12.5 ± 1.0 mg/g in WT, TG^{SER}, TG^{CSQ} and TG^{CxS}, respectively (n=7, $P<0.05$). Stained tissue sections revealed an enhanced interstitial fibrosis in ventricles of both TG^{CSQ} and TG^{CxS}. Functional analysis was performed on isolated working heart preparations. Contractility (+dP/dt) was depressed in TG^{CxS} (1378 ± 144 mmHg/s) compared to WT, TG^{SER} and TG^{CSQ} (2461 ± 119, 3644 ± 161 and 1879 ± 105 mmHg/s, respectively, n=6, $P<0.05$). We conclude that the additional overexpression of SERCA2a failed to rescue the impaired cardiac phenotype of CSQ transgenic mice.

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Ablation of Histidine-Rich Calcium Binding Protein (HRC) Results in Severe Cardiac Hypertrophy and Dysfunction in Pressure Overload Model

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HRC is a high capacity Ca^{2+} binding protein located in the lumen of sarcoplasmic reticulum (SR). Previously, we had shown that HRC knock-down not only led to enhanced Ca^{2+} release and Ca^{2+} uptake in SR, but exacerbated cardiac function after transverse aortic constriction (TAC), suggesting its important role in Ca^{2+} cycling and cardiac function. HRC-KO mice were generated and used for the present study. Pressure overload induced by TAC in the KO mice heart resulted in severe cardiac hypertrophy with 27% increase of heart weight (HW) per body weight (BW) ratio and 25% increase of tibia length per BW ratio as compared to WT TAC mice. HRC KO mice also showed decreased fractional shortening (FS) by 37%, increased TGF-β expression by 2 folds, severe cardiac fibrosis and highly increased number of TUNEL positive signals in heart tissue compared to WT TAC mice. The electrocardiogram (ECG) study showed shorter RR interval, faster heart rate and decreased R amplitude in HRC KO TAC mice. The incidence of arrhythmia was significantly increased in HRC KO mice after **intraperitoneal injection of caffeine (120 mg/kg BW) and epinephrine (2 mg/kg BW),indicating that HRC KO mice are more susceptible to epinephrine and caffeine injection, consistent with the previous report (**Jaehnig **et al.** Mol. Cell. Biol.**, 2006).** The survival rate of HRC KO mice was significantly decreased after TAC. Taken together, our results suggest that ablation of HRC could lead to altered SR Ca^{2+} cycling and deterioration of cardiac function under pathological condition. (Supported by Korea MEST NRF Grant (20110002144), the 2011 GIST System Biology Infrastructure Establishment Grant and KISTI-KREONET).

Cardiac Muscle III

2820-Pos Board B590

Myofilament Phosphorylation and Function in Diastolic Heart Failure

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This study aimed to compare alterations in phosphorylation and function of sarcomeric proteins in diastolic heart failure (DHF) vs. normal myocardium under two conditions of tissue procurement: beating-heart biopsy and postmortem tissue sampling. Left ventricular tissue samples were procured from normal (CTRL) or old dogs made hypertensive by renal wrapping (DHF), by either taking biopsies of the beating heart (n=7) or excising tissue postmortem (n=8). Isolated permeabilized cardiomyocytes were attached to a force transducer and passive tension ($F_{passive}$) was measured between 1.8 and 2.4 μm, calcium sensitivity (pCa_{50}) at 2.2 μm sarcomere length. Phosphorylation of myofilament proteins was assessed using the SYPRO-Ruby (total protein) / Pro-Q Diamond (phosphoprotein) system. Expression of total or phosphorylated protein, including titin-PEVK, cTnI, and PKCα was also quantified by immunoblot. Postmortem tissues and biopsy samples showed similar changes in DHF vs. CTRL, including increased phospho-PEVK, phospho-PKCα, and $F_{passive}$, but decreased titin N2BA:N2B isoform ratio and reduced phosphorylation of total titin, cMyBPC, cTnT, cTnI and cTnI (S23/24). Differences were apparent in terms of lowered pCa_{50} in postmortem DHF, but increased pCa_{50} in biopsy DHF. Reduced MLC2 phosphorylation in DHF vs. CTRL was observed in biopsies, but not in postmortem tissues. A degradation form of titin (T2) was more abundant in postmortem tissues than in biopsies. We conclude that this DHF model is characterized by titin-isoform shift towards the stiff N2B isoform, a deficit in total titin phosphorylation, but increases in PKCα-dependent phosphorylation of titin-PEVK-domain and cardiomyocyte $F_{passive}$, as well as altered pCa_{50}. For a few parameters, postmortem and beating-heart samples differed in the direction of change in DHF vs. CTRL, particularly regarding Ca^{2+}-sensitivity. However, most parameters changed in the same direction, suggesting the cellular events associated with death alter sample properties to a modest degree.

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Troponin Phosphorylation Crosstalk

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The troponin (Tn) complex is a critical regulatory and central integrative hub for post-translational modifications (PTM) within the thin filament. There are a number of Tn phosphorylations; however, β-adrenergic-induced protein kinase A (PKA) phosphorylation of cardiac troponin I (TnI) acts as the major sarcomeric modulator of function. Traditionally, individual phosphorylation sites have been studied, yet simultaneous phosphorylation of multiple sites occurs in the heart. To fully understand the molecular mechanisms underlying cardiac muscle regulation, Tn phosphorylation must be studied as integrated events. As an initial step towards understanding Tn crosstalk, we employed a two-model phosphomimetic approach using cardiac human Tn. We studied AMP-activated protein kinase (AMPK) TnI phosphorylation (TnI-S150D) and protein kinase C (PKC) cardiac troponin T (TnT) phosphorylation (TnT-T284E) to investigate the influence of these modifications on the effects exerted by TnI PKA phosphorylation (TnI-S23/24D). The combinatorial effects of these phosphorylation events on TnI-S23/24D was determined by measuring troponin C calcium binding properties in the reconstituted thin filament. Calcium dissociated from TnI-S23/24D thin filaments at 368/s compared to 103/s for wild-type (WT). Results demonstrate that TnI-S150D alone decreases calcium dissociation by 50%, while TnI-S150D integration with TnI-S23/24D blunted TnI-S23/24D kinetics by 15%, demonstrating the intramolecular crosstalk of TnI-S150D to alter the effects of TnI PKA phosphorylation. On the other hand, TnT-T284E calcium dissociation was identical to WT, but when present with TnI-S23/24D calcium dissociation was enhanced 136% compared to TnI-S23/24D alone. This demonstrates an intermolecular influence of TnT phosphorylation on TnI PKA effects. Overall, these findings demonstrate the combinatorial effects of Tn site-specific phosphorylation by AMPK and PKC crosstalk to affect the function of TnI PKA-induced phosphorylation through both intra- and inter- molecular mechanisms. These data further highlight the importance of understanding the role of integrated Tn PTM crosstalk on cardiac contractile regulation.

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Target Specific Phosphorylation of Cardiac Troponin I and Sex Dimorphic Myofilament Function in R403Q Mice

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Male mice expressing an autosomal dominant mutation in alpha-myosin heavy chain (R403Q) develop hypertrophic cardiomyopathy characterized by progressive left-ventricular dilation and cardiac dysfunction, whereas females do not. We hypothesize that this sexual dimorphism exists on multiple levels, including cellular metabolism regulation, post-translational modulation of contractile proteins and mechanical functions of contractile fibers. Hearts from wild-type (WT) and R403Q male and female animals with established disease were analyzed for myosin heavy chain (MyHC) isoform expression, total troponin I (TnI) phosphorylation, site-specific TnI phosphorylation, AMPKα expression, and AMPK activity. Ca2+-sensitive tension development was measured in demembranated cardiac trabeculae. R403Q male and female mice demonstrated progressive cardiac hypertrophy beginning at 4 months of age. β-MyHC expression increased in male and female R403Q mice. Total phosphorylation of TnI expression was independent of sex and R403Q mutation. No difference was found in phospho-TnI-Ser22/23 in R403Q animals relative to WT controls, however R403Q females had increased expression of phospho-TnI-Ser150 relative to WT counterparts. R403Q males showed increased expression of AMPKα, however had decreased AMPK activity relative to WT counterparts. R403Q females had reduced AMPKα expression but unchanged AMPK activity. The R403Q mutation does not affect Ca2+-sensitive tension development in demembranated cardiac trabeculae from males; female R403Q cardiac trabeculae were more sensitive to Ca2+ than WT controls. In conclusion, R403Q mice display hypertrophy with increased expression of hypertrophy marker β-MyHC. There exists a sexually dimorphic pattern of TnI